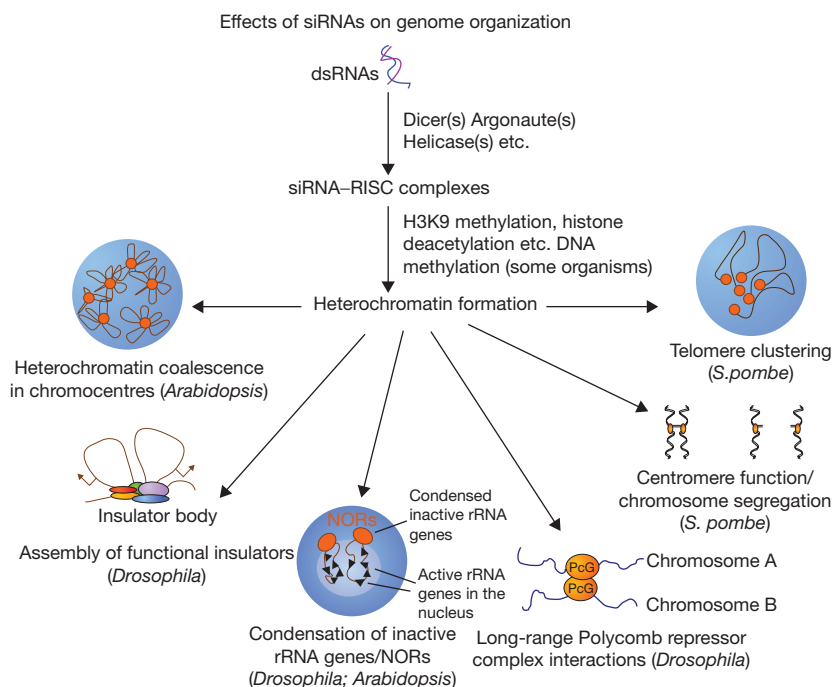# Heterochromatin: condense or excise

Craig Pikaard and Olga Pontes

In eukaryotes, condensing chromosomal DNA into heterochromatin is important for gene silencing and proper chromosome segregation. A recent study suggests a function for heterochromatin in protecting highly repeated genes and satellite DNA against excision caused by recombination or by joining of free DNA ends following DNA damage.

Chromosomes are made of chromatin — a combination of DNA and tightly associated proteins, including histones. The structure of chromatin in the genome is not uniform and this is readily apparent on examining nuclei stained with DNA-binding dyes under a light microscope. For example, during interphase, metazoan nuclei typically display regions of intensely stained heterochromatin that stand out prominently against a background of diffuse euchromatin. Single-copy and low-copy number genes that give rise to most of the cellular mRNAs are found primarily in euchromatin[1]. In contrast, the heterochromatic fraction of the genome corresponds to highly condensed chromosomal regions, from which few mRNAs are produced and where there is an abundance of repetitive genes and DNA elements (such as satellite repeats and transposons). On page 25 of this issue, Peng and Karpen[2] provide important insights into the functional consequences of heterochromatin formation by showing that mutations that disrupt the assembly of heterochromatin perturb the spatial distribution, organization and stability of tandem-repeat arrays within the *Drosophila* genome.

Peng and Karpen analysed ribosomal RNA (rRNA) genes that are clustered in loci known as nucleolus organizer regions (NORs) on the X and Y sex chromosomes, and two families of repeats, either clustered on two autosomes or found adjacent to the NOR on the X chromosome. The authors found that segments of rRNA genes and satellite repeat arrays become dispersed in *Drosophila* mutants that are defective in the histone H3 Lys 9 (H3K9) methyltransferase Su(var)3-9, in HP1 (heterochromatin protein 1, also known as Su(var)2-5; binds to methylated H3K9) or in several genes involved in the RNA interference (RNAi) pathway. The dispersion of repeats is particularly evident in polytene nuclei of salivary glands

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Figure 1 RNAi-dependent effects on heterochromatin and genome architecture. Double-stranded RNAs (dsRNAs) generated by bidirectional transcription or the action of RNA-dependent RNA polymerases are cleaved into siRNAs by dicer endonucleases. siRNAs are then loaded into RNA-induced silencing complexes (RISC) that are characterized by the presence of an Argonaute family protein. RISC complexes are thought to mediate downstream events, including the transcriptional silencing of sequences matching siRNAs due to H3K9 methylation, histone deacetylation, DNA methylation (in organisms that methylate their DNA) and other modifications characteristic of heterochromatin. Examples in which the organization of the genome is affected by RNAi-related mechanisms include the clustering of telomeres and the establishment of functional kinetochores at centromeres in *S. pombe,* the long-range interaction of polycomb repressor complexes in *Drosophila*, the condensation of inactive rRNA genes into heterochromatic knobs or chromomeres at NORs in *Drosophila* and plants, the formation of insulator complexes that protect genes from the influence of flanking chromatin in *Drosophila* and the long-range association of heterochromatic sequences into chromocentres in *Arabidopsis*.

in which euchromatic and heterochromatic sequences have been endo-reduplicated to varying extents.

Because rRNA gene expression is the driving force for the assembly of nucleoli[3,4], one consequence of dispersing rRNA genes is the appearance of multiple nucleoli, instead of the single nucleolus typical of wild-type cells. Pursuing this observation, Peng and Karpen found evidence that rRNA genes and pericentromeric repeats are not simply decondensed in the Su(var) or RNAi mutants but instead, they seem to accumulate as extrachromosomal DNAs.

The accumulation of these extrachromosomal DNAs is partially suppressed by mutations in Ligase 4, a protein implicated in DNA damage repair mediated by non-homologous end joining. Collectively, these observations lead the authors to suggest a model whereby the RNAi machinery acts in heterochromatin formation at tandemly arrayed repeats, with the resulting chromatin condensation suppressing events that could excise potentially large sections of the arrays as extrachromosomal circles.

This work resonates with several previous studies of rRNA gene function and

organization in mammals and plants. Eukaryotes seem to have many more rRNA genes than are needed in a typical cell, such that only a fraction of the genes are expressed at any one time[5]. The active rRNA genes in both mammals and plants display typical marks of euchromatin, including histone H3K4 methylation and hyperacetylation of histones H3 and H4. In contrast, the silenced subset of rRNA genes in mammals and plants display typical marks of heterochromatin, including H3K9 methylation, histone hypoacetylation and DNA hypermethylation[6,7]. Mutations or chemicals that disrupt heterochromatin formation by inhibiting DNA methylation or histone deacetylation cause the decondensation of the NORs within the nucleus coincident with the derepression of the silenced rRNA genes[7,8]. One plant histone deacetylase that has a particularly important role in NOR condensation is HDA6 (refs 8, 9), a gene which has also been identified in genetic screens designed to identify important players in small interfering RNA (siRNA)-directed DNA methylation and transcriptional gene silencing[10]. The latter observation has suggested that siRNAs might function in rRNA gene silencing and condensation of NORs into heterochromatin. Although not specifically addressing the issue of rRNA gene expression, the genetic evidence from the work of Peng and Karpen that rRNA genes lose their H3K9 methylation and become dispersed on mutation of Dicer-2 (the endonuclease responsible for siRNA formation in flies), or other siRNA pathway proteins[2], now provides much stronger evidence that siRNAs are somehow involved in heterochromatin formation at NORs.

The most intriguing aspect of Peng and Karpen's work is the evidence that *Drosophila* NORs are not simply decondensed on depleting the siRNA machinery or heterochromatic marks — as is thought to be the case in *Arabidopsis*. Instead, the evidence points to recombination or DNA damage-repair events (such as the joining of free DNA ends caused by DNA breakage or the stalling of replication forks) that lead to the production of extrachromosomal arrays of rRNA genes and satellite repeats. They hypothesize that one function of heterochromatin is to suppress such events. An apparent parallel in budding yeast is that the NAD-dependent histone deacetylase SIR2 is also known to suppress recombination among rRNA genes[11], suggesting that heterochromatin-mediated suppression of recombination may be a general strategy employed at eukaryotic NORs

Peng and Karpen indicate that nuclear and nucleolar volumes increase proportionally in *Su(var)* and RNAi mutants relative to wild-type cells, suggesting that siRNA-mediated heterochromatin formation has widespread effects on genome organization, a hypothesis for which there is growing consensus (see Fig. 1). For instance, mutations in *Drosophila* RNAi pathway proteins have been shown to reduce H3K9 methylation and cause the dramatic mislocalization of heterochromatin proteins HP1 and HP2 along the entire length of polytene salivary gland chromosomes[12]. The RNAi machinery of flies also affects chromatin insulator function[13], as well as long-range chromosome interactions mediated by the association of polycomb group repressor complexes[14]. In *Schizosaccharomyces pombe* mutants defective for Rdp1, an RNA-dependent RNA polymerase required for siRNA production, telomere clustering and mitotic chromosome segregation are disrupted[15], and in plants, RNA polymerase IV mutants that abolish nuclear siRNA production also disrupt the coalescence of heterochromatic sequences within chromocenters[16]. No doubt these studies are merely revealing the tip of the iceberg with regard to the direct and indirect roles of siRNAs in organizing genomic chromatin.

These studies lead to a number of predictions and questions. Because extrachromosomal DNAs presumably lack functional centromeres, excised rRNA genes and satellite repeats should be prone to loss during mitosis or meiosis. Whether repeats are actually lost on transmission of chromosomes through the germline has not yet been addressed. Moreover, pursuing this prediction is likely to be difficult for technical reasons — due to the repetitive nature and high degree of sequence similarity among repeats, and the large size of the loci in question — but it is nonetheless an intriguing and ultimately testable hypothesis. Likewise, it would be interesting to know the sizes and conformation of extrachromosomal repeats generated in mutants defective for heterochromatin formation, which should be possible using pulsed-field gel electrophoresis and may yield insights into the breakpoints and origins of the extrachromosomal DNAs. Finally, it is not yet clear whether there are siRNAs that correspond to rRNA genes or satellite repeats and whether they are causally involved in heterochromatin formation at these loci. Identifying the relevant siRNAs and determining their mode(s) of action would certainly substantiate the genetic and cytological evidence in Peng and Karpen's intriguing new study. □

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